PB82-128158

Effect of Fluctuating Exposures on the Acute Toxicity of Ammonia to Rainbow Trout (Salmo gairdneri) and Cutthroat Trout (S. clarki)

Montana State Univ., Bozeman

Prepared for

Environmental Research Lab.
Duluth, MN

Oct 81

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/J-81-345	2. Journal Article
3. REC	PB82-128158
4. TITLE AND SUBTITLE Effect of Fluctuating Exposures on the Acute Toxicity of Ammonia to Rainbow Trout (Salmo gairdneri) and Cutthroat Trout (S. clarki)	5. REPORT DATE October 1981 6. PERFORMING ORGANIZATION CODE 317007
7. AUTHOR(S) Thurston, R. V., C. Chakoumakos, and R. C. Russo	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Fisheries Bioassay Laboratory Montana State University Bozeman, MT 59717	10. PROGRAM ELEMENT NO. 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS U.S. Environmental Protection Agency Environmental Research Laboratory-Duluth 6201 Congdon Boulevard Duluth, MN 55804	13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE

15. SUPPLEMENTARY NOTES
Water Res. 15: 911-917 (1981)

16. ABSTRACT

Acute toxicity bioassays in which fish were exposed to short-term cyclic fluctuations of ammonia were conducted on rainbow trout (Salmo gairdneri) and on cutthroat trout (S. clarki). Companion tests were also conducted in which test fish were subjected to ammonia at constant concentrations. The tests ranged in duration from 96 h to 4 weeks. Median lethal concentration (LC50) values in terms of both average and peak concentrations of ammonia for the fluctuating concentration tests were compared with LC50 values for the constant concentration (conventional) tests. Based on comparisons of total dose exposure, results showed that fish were more tolerant of constant concentrations of ammonia than of fluctuating concentrations. Fish subjected to fluctuating concentrations of ammonia at levels below those acutely toxic were subsequently better able to withstand exposure to higher fluctuating concentrations that fish not previously so acclimated.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

PRODUCT OF:
NATIONAL TECHNICAL INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS *(This Report)* UNCLASSIFIED	21. NO. OF PAGES 7
	20. SECURITY CLASS *(This page)* UNCLASSIFIED	22. PRICE

PB82 128158

Water Research Vol. 15, pp. 911 to 917, 1981
Printed in Great Britain. All rights reserved

0043-1354/81/070911-07$02.00/0
Copyright © 1981 Pergamon Press Ltd

EFFECT OF FLUCTUATING EXPOSURES ON THE ACUTE TOXICITY OF AMMONIA TO RAINBOW TROUT (*SALMO GAIRDNERI*) AND CUTTHROAT TROUT (*S. CLARKI*)

ROBERT V. THURSTON[1], CHARLES CHAKOUMAKOS[2] and ROSEMARIE C. RUSSO[1]

[1]Fisheries Bioassay Laboratory, Montana State University, Bozeman, MT 59717 and
[2]Department of Chemistry, University of Maine at Farmington, Farmington, ME 04938, U.S.A.

(*Received January* 1981)

Abstract—Acute toxicity bioassays in which fish were exposed to short-term cyclic fluctuations of ammonia were conducted on rainbow trout (*Salmo gairdneri*) and on cutthroat trout (*S. clarki*). Companion tests were also conducted in which test fish were subjected to ammonia at constant concentrations. The tests ranged in duration from 96 h to 4 weeks. Median lethal concentration (LC 50) values in terms of both average and peak concentrations of ammonia for the fluctuating concentration tests were compared with LC 50 values for the constant concentration (conventional) tests. Based on comparisons of total dose exposure, results showed that fish were more tolerant of constant concentrations of ammonia than of fluctuating concentrations. Fish subjected to fluctuating concentrations of ammonia at levels below those acutely toxic were subsequently better able to withstand exposure to higher fluctuating concentrations than fish not previously so acclimated.

INTRODUCTION

Ammonia is a common component in the discharges from sewage treatment plants and many industrial processes. Published criteria for ammonia to protect aquatic life (EIFAC, 1970; U.S. EPA, 1977) are based on reported toxic effects of constant (fixed) concentrations of ammonia under acute or chronic conditions and do not consider the effect of short-term excursions in excess of these fixed concentrations.

Because of typical household water use patterns, demands on sewage treatment facilities include daily cycles with at least 2 peaks. Frequently the discharge patterns from the treatment facilities reflect this (Metcalf & Eddy Inc., 1972), and aquatic life in the receiving streams are subjected to daily fluctuations in ammonia concentrations in their environment as opposed to fairly steady or constant concentrations. Discharges of ammonia from some industrial plants may also evidence a daily or weekly pattern which reflects the industrial process involved. There are also cases of accidental spills in which aquatic animals are subjected to doses or single "peaks" of ammonia for short periods of time, often only a matter of a few minutes or hours. Although "fish kills" have been reported as a result of some of these accidental discharges, there is virtually no information available about the tolerance of fishes to such short-term "peaks" or doses.

The objective of the research reported here was to compare the acute toxicity of ammonia to fishes when they are exposed to cyclic fluctuations and concentration "peaks" as opposed to exposure at constant concentrations.

METHODS

A total of 14 continuous-flow bioassays were conducted, 10 of these on rainbow trout (*Salmo gairdneri*) from three different lots of fish and 4 on cutthroat trout (*S. clarki*) from a single lot. Prior to test, all of the fishes had been reared at the Fish Cultural Development Center, U.S. Fish and Wildlife Service (F & WS), Bozeman, MT. Because the fishes were from four different lots, the bioassays were grouped as four different series of tests (I–IV). At least one conventional bioassay was conducted in each test series, and one or more additional bioassay was conducted in which toxicant was intermittently added to the dilution water under a time-regulated, cyclic regime. The addition of toxicant in this manner resulted in concentrations of ammonia in the test tanks mounting to peaks or plateaux, each followed by a flushing period during which the concentrations dropped to or near zero.

All bioassays were conducted in fiberglass tanks (1.2 m dia.) of 350-l. capacity. One control tank was used in each bioassay, and in 11 of the bioassays there were 5 test tanks, each at different concentrations of toxicant solution. In the other 3 bioassays there were 4, 3, or 1 test tanks as noted below. Fish were selected and distributed at random from the pool of fish for any test series and among the tanks for any test. Ten fish were tested in each tank except for tests 614, 615, 632 and 633, in which 5 fish were tested in each tank. Prior to testing, fish were fed a F&WS hatchery production diet (Rangen Inc., Buhl, ID). Test fish were acclimated to the tanks for 24–48 h before the start of any test and were not fed during that time or during the next 96-h period; fish were fed in some of the tests after 96 h as described below in the Results section.

The source of the dilution water for the tests was a natural ground spring, characteristics of which are listed in Table 1; prior to test, fish had been reared in water from the same source. The dilution water was added to each tank at the rate of 5–6 l min^{-1} and toxicant (reagent grade NH_4Cl) solution was metered into each test tank at a different rate (<1–5 ml min^{-1}). The toxicant solution was added to the test tanks at the point of addition of the

Table 2. Results of 96-h acute toxicity bioassays

Test no.	Toxicant addition cycle (hours on/off)	Avg. fish size (g)	Temperature (°C) avg. (Range)	pH avg. (Range)	96-h LC 50 (95% C.I.) (Conventional LC 50)		96-h MC-LC 50 (95% C.I.) (Mean concentration LC 50)		96-h PC-LC 50 (95% C.I.) (Peak concentration LC 50)	
					($mg\,l^{-1}$ NH_3–N)	($mg\,l^{-1}$ NH_3)	($mg\,l^{-1}$ NH_3–N)	($mg\,l^{-1}$ NH_3)	($mg\,l^{-1}$ NH_3–N)	($mg\,l^{-1}$ NH_3)
					Series I—*Rainbow trout*					
580*	96/0	21	9.7 —†	7.86 (7.81–7.89)	31.6 (26.5–37.6)	0.500 (0.420–0.593)				
574‡	6/6	18	9.6 (9.3–9.8)	7.86 (7.79–7.97)			19.3 (16.9–22.1)	0.292 (0.257–0.332)	38.2 (33.4–43.8)	0.579 (0.510–0.658)
					Series II—*Rainbow trout*					
637	96/0	247	8.1 (7.9–8.3)	7.74 (7.72–7.80)	28.0 (24.8–31.6)	0.296 (0.261–0.335)				
635	6/6	230	8.3 (8.1–8.5)	7.74 (7.64–7.84)			22.3 (19.5–25.5)	0.221 (0.195–0.251)	40.4 (35.5–45.9)	0.401 (0.356–0.453)
639	6/6	293	8.5 (7.9–8.8)	7.71 (7.64–7.76)			20.7 NC§	0.214 NC	38.3 NC	0.395 NC
636	12/12	244	8.1 (8.0–8.3)	7.72 (7.59–7.80)			19.8 (17.7–22.1)	0.176 (0.167–0.184)	40.1 (36.2–44.4)	0.356 (0.343–0.371)
634	6/18	230	8.2 (8.1–8.4)	7.72 (7.61–7.84)			10.5 (7.2–15.3)	0.099 (0.067–0.149)	40.0 (28.6–56.0)	0.377 (0.266–0.536)
638¶	2.5/93.5	242	8.2 —	7.73 —					>45.3	>0.476
					Series III—*Rainbow trout*					
633‖	96/0	2596	7.9 (7.7–8.0)	7.62 (7.52–7.71)	21.6 (17.6–26.6)	0.163 (0.139–0.190)				
632‖	6/6	2080	7.7 (7.5–8.0)	7.67 (7.60–7.83)			17.0 (14.4–20.0)	0.145 (0.122–0.173)	34.2 (29.0–40.3)	0.292 (0.246–0.347)
					Series IV—*Cutthroat trout***					
615‖	96/0	3.6	10	7.7	29.1 (25.3–33.4)	0.327 (0.284–0.375)				
617	96/0	4.1	10	7.7	26.3 (23.7–29.2)	0.296 (0.266–0.328)				
614‖	12/12	3.6	10	7.7			17.3 (15.4–19.4)	0.194 (0.173–0.217)	34.7 (30.8–39.0)	0.389 (0.346–0.438)
616	12/12	4.1	10	7.7			19.3 (18.4–20.3)	0.217 (0.206–0.228)	38.8 (36.9–40.8)	0.435 (0.414–0.458)

* 4 test tanks.
† — = All values measured were the same, therefore no range is given.
‡ 3 test tanks.
§ NC = Not calculable.
¶ 1 test tank.
‖ 5 fish/tank; all other other tests 10 fish/tank.
** pH and temperature values for Series IV tests are estimates.

remaining 9 fish all evidenced stress; 3 of these were floating on their backs, the other 6 either swimming on one side or rolling from side to side, and all were having difficulty breathing. These symptoms are typical of acute ammonia poisoning. The surviving 9 fish were immediately transferred to ammonia-free water, and within 30 min the 3 fish which had been floating on their backs had died; the remaining 6 fish gradually recovered their equilibrium, and all appeared to be fully recovered by the close of day 1. There were no further mortalities during the balance of the 96-h test period.

Tests continued beyond 96 h

Five of the bioassays were continued beyond the initial 96-h period, either maintaining the initial test protocol or with some variation to it.

Test 639 (6-on/6-off) was continued until day 28; there were no mortalities at the highest test tank concentration after the third cycle (during day 2) although there was one mortality each at the next two lower concentrations, one of these on day 9 and the other on day 24. The 28 day MC-LC 50 and PC-LC 50 values were the same as those for 96 h.

Test 617, a conventional bioassay, was continued through day 14 and mortalities continued to occur at all tank concentrations; no asymptotic LC 50 value was reached during the test period. The 14-day LC 50 values and their confidence intervals were 0.241 (0.229–0.254) mg l^{-1} NH_3 and 21.5 (20.4–22.6) mg l^{-1} NH_3–N. These values are 18–19% lower than the 96-h LC 50 values.

Test 616 (12-on/12-off) was also continued through day 14; however, toxicant addition was discontinued after 96 h and the fish were maintained in the test tanks without ammonia through day 14. There were no mortalities during the "recovery" period.

In test 632, following the initial 96-h cyclic period (6-on/6-off), toxicant was again raised to the peak concentrations and maintained at that level for an additional 96-h period. Four of the 5 fish at the highest tank concentration (0.37 mg l^{-1} NH_3) had expired during the first 30 h of the cyclic period; the sole survivor at 96 h also survived the sustained peak concentration for the additional 96-h period. There had been no mortalities at the next two lower concentrations (peak concentrations 0.20 and 0.15 mg l^{-1} NH_3) during the initial 96-h test period, but there was one mortality in each of these tanks within 24 h of beginning the sustained peak concentrations; the remainder of the fish in these two tanks survived the additional 96-h test period.

Test 574 (6-on/6-off) was continued with the same toxicant addition regime for 7 days beyond 96 h, but at 96 h the fish from the lower two concentrations (average peak concentrations 0.11 and 0.39 mg l^{-1} NH_3) were transferred to the highest concentration tank (average peak concentration 0.74 mg l^{-1} NH_3). Two of the 10 fish at the highest concentration and all 10 fish from each of the lower two concentrations had survived the first 96-h test period. Following transfer to the highest concentration tank 5 of the 10 fish from the lowest concentration died during the next 3 cycles; the remaining 5 fish from the lowest concentration and all 10 of the fish transferred from the middle concentration survived the additional 7-day period. The 2 fish which had already survived 96-h at the highest concentration also survived the next 7 days. Observations after day 5 included the following. During the cycle peaks, between 8–14 of the 17 fish under observation evidenced equilibrium loss, ranging from minor disorientation to floating upside down. By day 10, all but 2 fish were able to recover much of their equilibrium during the time between ammonia concentration peaks, suggesting some adaptation to the ammonia fluctuation regime. Food pellets were provided *ad libitum* starting on day 9; approximately half of the fish, including one of the fish which swam on its back, evidenced interest in eating. On day 11, toxicant addition was discontinued and fish were maintained and observed for an additional 7 days. Within 24 h most of the fish were swimming upright, although occasional lapses would occur during resting phases; this behavior continued through day 15. By day 17 all fish were feeding vigorously, appeared normal, and no loss of equilibrium was observed at any time. The test was terminated on day 18.

Mortality data treatment

In computing the MC-LC 50 and PC-LC 50 values reported above, we have taken certain liberties, as described, in our choice of data input to a conventional method for computing LC 50 values. The statistical validity of the MC-LC 50 and PC-LC 50 values we obtained has not been tested. Accordingly, the 96-h test data were further scrutinized in the following manner.

For each of the fluctuating tests (except Test 638 which had a single peak) we selected the test tank with the lowest mean ammonia concentration in which at least 50% of the test fish died. The mean concentration in that tank was compared against the LC 50 for the corresponding conventional test. Except for Test 632, the mean of the fluctuating ammonia concentrations at which 50% or more of the fish died was lower than the LC 50 value for the conventional test used for comparison (Table 3). Similarly, for each fluctuating test the tank with the highest peak ammonia concentration at which 50% or more of the test fish survived was selected, and the average peak concentration in the tank was compared against the conventional LC 50. In all cases the peak concentration values were greater than the LC 50 values from the corresponding conventional tests used for comparison (Table 3). Because two conventional tests had been conducted in Series IV, yielding two slightly different LC 50 values, the lower LC 50 value was used for comparison against the mean tank concentrations for the fluctuating tests in that series, and the higher LC 50 value was used for comparison against the

Table 3. Comparisons of conventional test LC 50 values against mean concentrations with ≥ 50% mortality and peak concentrations with ≥ 50% survival in fluctuating tests. (Ammonia concentrations expressed as mg l^{-1} NH_3)

		Comparison of mean concentrations				Comparison of peak concentrations		
Test no.	Corresponding conventional test LC 50*	Lowest test tank concentration with ≥ 50% mortality	% Mortality	Ratio of tank concentration to conventional LC 50	Highest test tank concentration with ≥ 50% mortality	% Survival	Ratio of tank concentration to conventional LC 50
574	0.500	0.372	80	0.74	0.387	100	1.10
635	0.296	0.275	60	0.93	0.325	80	1.10
639	0.296	0.214	50	0.72	0.310	100	1.05
636	0.296	0.268	100	0.91	0.339	70	1.15
634	0.296	0.096	50	0.32	0.359	50	1.21
632	0.163	0.183	80	1.12	0.199	100	1.22
614	0.296	0.248	100	0.84	—	—	—
614	0.327	—	—	—	0.374	60	1.14
616	0.296	0.256	100	0.86	—	—	—
616	0.327	—	—	—	0.382	90	1.17

* From Table 2. See Discussion for explanation of two different LC 50 values used for comparisons with Tests 614 and 616.

peak tank concentrations. The comparisons followed the trend obtained for the other test comparisons.

Comparison of results with those reported elsewhere

Brown *et al.* (1969) tested rainbow trout in static tests in which fish were moved back and forth between tanks in which the ammonia concentrations were 0.5 and 1.5 times a previously determined 48 h LC 50. If fish were transferred on an hourly basis, the median period of survival was reported to be the same as that for constant exposure (> 700 min); if the fish were transferred at 2-h intervals, the median survival time was reported to be less (370 min). The results of the tests in which the fish were transferred at 2-h intervals are in keeping with our findings; i.e. the toxic effects from exposure to fluctuating concentrations of ammonia are greater than those from exposure to a constant concentration, if the mean "dose" exposure is the same in both cases.

CONCLUSIONS

1. Fish were better able to withstand a fixed concentration of ammonia over a 96-h time period than they were fluctuating concentrations having mean values comparable to that of the fixed concentration.
2. Fish were able to withstand short-term excursions slightly above acutely toxic (96-h LC 50) concentrations of ammonia without apparent longer-term effects, provided that those excursions were accompanied by compensatory respite periods below the acutely toxic concentrations.
3. Fish subjected for 96-h periods to cyclic pulses of ammonia at concentrations below those acutely toxic were better able to withstand subsequent exposure to acutely toxic pulses than were fish not previously so "acclimated".
4. Among the rainbow trout tested, the larger fish (> 2 kg) were more vulnerable to acutely toxic concentrations of ammonia than were the smaller fish (20–300 g).

Acknowledgements—This research was funded by the U.S. Environmental Protection Agency, Environmental Research Laboratory-Duluth, Research Grant No. R805636. Elizabeth L. Meyn and Kenneth C. Wang assisted with water chemistry analyses. Martin A. Hamilton, MSU Statistical Laboratory, contributed helpful comments with regard to the data analysis.

REFERENCES

American Public Health Association (1976) *Standard Methods for the Examination of Water and Wastewater*, 14th Edition, 1193 pp. American Public Health Association, American Water Works Association, and Water Pollution Control Federation, Washington, DC.

Brown V. M., Jordan D. H. M. & Tiller B. A. (1969) The acute toxicity to rainbow trout of fluctuating concentrations and mixtures of ammonia, phenol and zinc. *J. Fish Biol.* **1**, 1–9.

Emerson K., Russo R. C., Lund R. E. & Thurston R. V. (1975) Aqueous ammonia equilibrium calculations: effect of pH and temperature. *J. Fish. Res. Bd Can.* **32**, 2379–2383.

European Inland Fisheries Advisory Commission (1970) Water quality criteria for European freshwater fish. Report on ammonia and inland fisheries. EIFAC Tech. Paper No. 11, 12 pp; also in *Water Res.* **7**, 1011–1022 (1973).

Hamilton M. A., Russo R. C. & Thurston R. V. (1977) Trimmed Spearman–Karber method for estimating median lethal concentrations in toxicity bioassays. *Envir. Sci. Technol.* **11**, 714–719. *Correction* **12**, 417 (1978).

Metcalf & Eddy Inc. (1972) *Wastewater Engineering: Collection, Treatment, Disposal*, 782 pp. McGraw-Hill, New York, NY.

Thurston R. V., Russo R. C. & Emerson K. (1979) Aqueous ammonia equilibrium—Tabulation of percent un-ionized ammonia, 427 pp. EPA Ecol. Res. Ser. EPA-600/3-79-091. U.S. Environmental Protection Agency, Office of Research and Development, Environmental Research Laboratory-Duluth, MN.

Thurston R. V., Russo R. C. & Smith C. E. (1978) Acute toxicity of ammonia and nitrite to cutthroat trout fry. *Trans. Am. Fish. Soc.* **107**, 361–368.

U.S. Environmental Protection Agency (1974) Methods for chemical analysis of water and wastes. EPA-625/6-74-003. U.S. Environmental Protection Agency, Washington, DC.

U.S. Environmental Protection Agency (1977) Quality criteria for water, 256 pp. Office of Water and Hazardous Materials, U.S. Environmental Protection Agency, Washington, DC.

Willingham W. T., Colt J. E., Fava J. A., Hillaby B. A., Ho C. L., Katz M., Russo R. C., Swanson D. L. & Thurston R. V. (1979) Ammonia. In *A Review of the EPA Red Book: Quality Criteria for Water* (Edited by Thurston R. V., Russo R. C., Fetterolf C. M. Jr, Edsall T. A. & Barber Y. M. Jr), pp. 6–18. Water Quality Section, American Fisheries Society, Bethesda, MD.

EPA-600/J-81-345
Journal Article

Water Research Vol. 15, pp. 911 to 917, 1981
Printed in Great Britain. All rights reserved

0043-1354/81/070911-07$02.00/0
Copyright © 1981 Pergamon Press Ltd

EFFECT OF FLUCTUATING EXPOSURES ON THE ACUTE TOXICITY OF AMMONIA TO RAINBOW TROUT (*SALMO GAIRDNERI*) AND CUTTHROAT TROUT (*S. CLARKI*)

ROBERT V. THURSTON[1], CHARLES CHAKOUMAKOS[2] and ROSEMARIE C. RUSSO[1]

[1]Fisheries Bioassay Laboratory, Montana State University, Bozeman, MT 59717 and
[2]Department of Chemistry, University of Maine at Farmington, Farmington, ME 04938, U.S.A.

(*Received January* 1981)

Abstract—Acute toxicity bioassays in which fish were exposed to short-term cyclic fluctuations of ammonia were conducted on rainbow trout (*Salmo gairdneri*) and on cutthroat trout (*S. clarki*). Companion tests were also conducted in which test fish were subjected to ammonia at constant concentrations. The tests ranged in duration from 96 h to 4 weeks. Median lethal concentration (LC 50) values in terms of both average and peak concentrations of ammonia for the fluctuating concentration tests were compared with LC 50 values for the constant concentration (conventional) tests. Based on comparisons of total dose exposure, results showed that fish were more tolerant of constant concentrations of ammonia than of fluctuating concentrations. Fish subjected to fluctuating concentrations of ammonia at levels below those acutely toxic were subsequently better able to withstand exposure to higher fluctuating concentrations than fish not previously so acclimated.

INTRODUCTION

Ammonia is a common component in the discharges from sewage treatment plants and many industrial processes. Published criteria for ammonia to protect aquatic life (EIFAC, 1970; U.S. EPA, 1977) are based on reported toxic effects of constant (fixed) concentrations of ammonia under acute or chronic conditions and do not consider the effect of short-term excursions in excess of these fixed concentrations.

Because of typical household water use patterns, demands on sewage treatment facilities include daily cycles with at least 2 peaks. Frequently the discharge patterns from the treatment facilities reflect this (Metcalf & Eddy Inc., 1972), and aquatic life in the receiving streams are subjected to daily fluctuations in ammonia concentrations in their environment as opposed to fairly steady or constant concentrations. Discharges of ammonia from some industrial plants may also evidence a daily or weekly pattern which reflects the industrial process involved. There are also cases of accidental spills in which aquatic animals are subjected to doses or single "peaks" of ammonia for short periods of time, often only a matter of a few minutes or hours. Although "fish kills" have been reported as a result of some of these accidental discharges, there is virtually no information available about the tolerance of fishes to such short-term "peaks" or doses.

The objective of the research reported here was to compare the acute toxicity of ammonia to fishes when they are exposed to cyclic fluctuations and concentration "peaks" as opposed to exposure at constant concentrations.

METHODS

A total of 14 continuous-flow bioassays were conducted, 10 of these on rainbow trout (*Salmo gairdneri*) from three different lots of fish and 4 on cutthroat trout (*S. clarki*) from a single lot. Prior to test, all of the fishes had been reared at the Fish Cultural Development Center, U.S. Fish and Wildlife Service (F & WS), Bozeman, MT. Because the fishes were from four different lots, the bioassays were grouped as four different series of tests (I–IV). At least one conventional bioassay was conducted in each test series, and one or more additional bioassay was conducted in which toxicant was intermittently added to the dilution water under a time-regulated, cyclic regime. The addition of toxicant in this manner resulted in concentrations of ammonia in the test tanks mounting to peaks or plateaux, each followed by a flushing period during which the concentrations dropped to or near zero.

All bioassays were conducted in fiberglass tanks (1.2 m dia.) of 350-l. capacity. One control tank was used in each bioassay, and in 11 of the bioassays there were 5 test tanks, each at different concentrations of toxicant solution. In the other 3 bioassays there were 4, 3, or 1 test tanks as noted below. Fish were selected and distributed at random from the pool of fish for any test series and among the tanks for any test. Ten fish were tested in each tank except for tests 614, 615, 632 and 633, in which 5 fish were tested in each tank. Prior to testing, fish were fed a F&WS hatchery production diet (Rangen Inc., Buhl, ID). Test fish were acclimated to the tanks for 24–48 h before the start of any test and were not fed during that time or during the next 96-h period; fish were fed in some of the tests after 96 h as described below in the Results section.

The source of the dilution water for the tests was a natural ground spring, characteristics of which are listed in Table 1; prior to test, fish had been reared in water from the same source. The dilution water was added to each tank at the rate of 5–6 l min^{-1} and toxicant (reagent grade NH_4Cl) solution was metered into each test tank at a different rate (<1–5 ml min^{-1}). The toxicant solution was added to the test tanks at the point of addition of the

Table 1. Chemical characteristics of the dilution water used in bioassays. (All values are mg l^{-1} unless otherwise noted)

Al	<1	Ni	<0.005
As	0.0012	Pb	<0.015
Ca	52.1	Se	0.00085
Cd	<0.005	Zn	0.01
Cr	<0.005	Cl^-	0.16
Cu	0.007	F^-	0.35
Fe	0.004	PO_4^{3-}	0.05
Hg	<0.00005	SO_4^{2-}	17.2
K	0.82	Total Organic Carbon	3.3
Mg	16.7	S.E.C., μS 25°C	340
Mn	0.002	Turbidity, NTU	1.6
Na	2.5		

dilution water and mixing was achieved by the plunging action of the incoming dilution water. In a preliminary test to confirm that mixing of the toxicant solution into the dilution water was complete, replicate samples were taken from each test tank at several locations, including different depths, 20 cm and more from the point of impact. Results showed no significant difference in ammonia concentration among these samples from any one tank at any one time.

In the bioassays with fluctuating concentrations of ammonia, the toxicant metering pumps were controlled by an automatic timing device which regulated the pumps in cycles of 6 h on/6 h off, 12-on/12-off, or 6-on/18-off. At the start of each bioassay the test tanks began at zero ammonia concentration; the peak concentration for each tank was effectively reached between 6 and 7 h from the start of the "on" phase. The pattern for the concentration cycles for the tanks in one of the tests (Test 574) is illustrated by the plots in Fig. 1; this pattern is representative. Five of the tests were continued beyond 96 h, either continuing the initial toxicant addition protocol or with some variation to it. The modifications for these tests after 96 h are mentioned individually where the test results are discussed below.

Chemical analyses were performed on water in the test tanks using the following methods. Total ammonia–nitrogen (NH_3–N) was determined using the nesslerization method from APHA (1976) and nitrite–nitrogen (NO_2–N) by the method of U.S. EPA (1974). Un-ionized ammonia (NH_3) concentrations were calculated from total ammonia concentrations using the formulas of Emerson *et al.* (1975) and table of Thurston *et al.* (1979). Oxygen was measured using a dissolved oxygen meter (Yellow Springs Instrument Co. Model 54) standardized by comparison with results of a Winkler test (APHA, 1976) on water from one of the tanks at each time of monitoring; phenylarsine oxide was substituted for sodium thiosulfate in the Winkler tests. Temperature was measured with a calibrated mercury thermometer, and pH with a digital meter (Beckman Phasar-I). Average pH values were obtained by use of a computer program which converted each measurement to the hydrogen ion concentration, averaged these, then reconverted the average to pH units. Alkalinity, hardness and nitrate–nitrogen (NO_3–N) were determined according to analytical procedures described in APHA (1976). Colorimetric measurements were made using a Varian Model 635 ultraviolet-visible spectrophotometer. During each 96-h test in Series I–III, dissolved oxygen, pH and temperature were measured in each tank on each of 3 separate days, and hardness, alkalinity, nitrate, and nitrite were measured at least once in each tank; for tests lasting longer than 96 h, dissolved oxygen, pH, and temperature were measured in each tank 3 times a week and other variables were measured weekly. During the Series IV tests no chemical measurements were made except for total ammonia; the reported pH and temperature values are estimates based on measurements made during other ammonia toxicity tests which were being conducted on approximately the same dates, using dilution water from the same source.

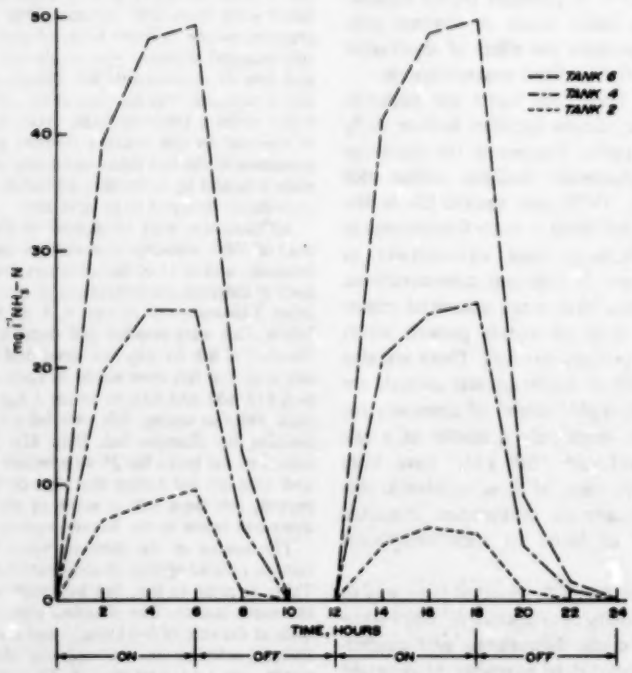

Fig. 1. Ammonia concentration profile for Test 574.

All 96-h bioassays in any series were conducted within a 4-week time period, and in one series a 6-on/6-off test was repeated after 2 weeks to test reproducibility. Mortality observations were made at 2–4-h intervals during the first 24 h of each test and at 6–12-h intervals thereafter. There was one control fish mortality on day 18 in Test 639; there were no control mortalities in any of the other tests. Fish were weighed individually at time of mortality observation or at the conclusion of each test.

LC 50 values and their 95% confidence intervals were calculated for all of the tests in terms of both total ammonia–nitrogen (NH_3–N) and un-ionized ammonia (NH_3) using the Trimmed Spearman–Karber Method (Hamilton *et al.*, 1977). For both the conventional and fluctuation tests, LC 50 values were calculated using averages (means) of measured tank ammonia concentrations; for the fluctuation tests, LC 50 values were also calculated using only peak concentrations. Peak and average concentrations for each of the fluctuation tests were obtained in the following manner.

Peak concentrations were obtained by measuring total ammonia in each tank within 15 min before the end of the toxicant-addition phases at least 4 times during any 4-day period. The averages of these measurements were used in calculating the LC 50 value in terms of peak concentration; for purposes of discussion in this paper, this non-conventional LC 50 so obtained will be referred to as the "peak concentration LC 50" (PC-LC 50).

Average (mean) concentrations for the cyclic bioassays were obtained by measuring total ammonia in each tank at 2-h intervals over at least one complete cycle and computing the mean concentration for each tank from these measured values. It is recognized that this incremental method of monitoring, as opposed to continuous monitoring, introduces a bias toward underestimating the true average concentration during the toxicant-addition phase of each cycle and overestimating the average during the flushing phase; it was assumed these biases offset each other.

In any one test there was some variation among the measured peak concentration values, greater in the low ammonia concentration tanks than in the high, and it was necessary to adjust for this. It was assumed that for any test the shapes of the ammonia concentration profiles in any one tank were reasonably similar from cycle to cycle (see Fig. 1) and that the range of variation among these profiles was comparable to that of the variation in measured peak values. The mean ammonia concentration for each tank during the entire test, then, was determined by computing the mean from the actual measurements during at least one complete on/off cycle and adjusting this by a factor derived from averaging the measured peak concentrations for that tank. The LC 50 value obtained, using the mean concentrations computed in this manner for each tank, will be referred to as the "mean concentration LC 50" (MC-LC 50).

RESULTS AND DISCUSSION

96-h Tests

Ninety-six-hour LC 50 values in terms of both total ammonia–nitrogen (NH_3-N) and un-ionized ammonia (NH_3) are reported in Table 2, along with pH, temperature and fish size for each test. Other measured water chemistry variables (and ranges) were as follows: dissolved oxygen, 8.7 (6.2–10.0) mg l^{-1}; NO_2–N, 0.00 (0.00–0.01) mg l^{-1}; NO_3–N, 0.12 (0.05–0.19) mg l^{-1}; alkalinity, 170 (161–180) mg l^{-1} as $CaCO_3$; hardness, 189 (157–197) mg l^{-1} as $CaCO_3$. Within the pH range over which the tests were conducted (7.6–7.9) the un-ionized species of ammonia is by far the principal toxic species (Thurston, submitted). For this reason comparisons among tests, in the discussion below, will be made on the basis of LC 50 values reported in terms of un-ionized ammonia (mg l^{-1} NH_3); any generalities so made would also be true for comparisons among tests made on the basis of LC 50 values in terms of total ammonia–nitrogen (mg l^{-1} NH_3–N).

The 96-h LC 50 values obtained for the conventional bioassays on cutthroat trout (0.30–0.33 mg l^{-1} NH_3) are in accord with earlier reported results on this same species (Thurston *et al.*, 1978). The 96-h LC 50 values obtained for the conventional bioassays on rainbow trout (0.30–0.50 mg l^{-1} NH_3) are also within the range of results reported elsewhere for this species as well (Willingham *et al.*, 1979), except for the results of Test 633. The 96-h LC 50 value of 0.16 mg l^{-1} NH_3 obtained in this test on 2–3 kg fish is lower than values heretofore reported for rainbow trout, but is, however, consistent with results of bioassays on other larger (older) rainbow trout (Thurston *et al.*, in preparation).

The 96-h MC-LC 50 values (mg l^{-1} NH_3) for the 6-on/6-off and 12-on/12-off cyclic tests ranged between 25 and 42% less than the LC 50 values obtained for the companion conventional bioassays, with one exception. The MC-LC 50 value for Test 632 on 2 kg rainbow trout was only 10% less than the LC 50 value for the companion conventional bioassay on comparable size fish. There seemed to be little difference in results within any series whether the cycles were 6-on/6-off or 12-on/12-off. The MC-LC 50 value for Test 634 with a cycle of 6-on/18-off was approximately half that of the other cyclic tests in that series. In this test the initial 6-h ammonia load period and 6-h flushing period were followed by a 12-h respite; i.e. for approximately half the test time the fish were in ammonia-free water.

The 96-h PC-LC 50 values (mg l^{-1} NH_3) also showed a consistency when compared with the results of the corresponding conventional bioassays. The PC-LC 50 values ranged from 16 to 39% higher than the LC 50 values, with one exception; once again the results of Test 632 fell outside the range of results for the other (smaller) fish. The PC-LC 50 value for this test on larger fish was 80% higher than the corresponding conventional LC 50. The preponderance of mortalities in the fluctuating tests occurred during the first and second cycle and all mortalities occurred by the close of the third cycle except for Test 635 in which a single mortality occurred during the sixth cycle.

One of the tests (Test 638) was conducted to observe the effect of a single lethal dose or "slug" of ammonia. Ten fish were placed in a single tank and subjected to a gradually increasing concentration of ammonia; ammonia addition was terminated at 2.5 h when the first mortality occurred. The concentration at that point had risen to 0.48 mg l^{-1} NH_3. The

Table 2. Results of 96-h acute toxicity bioassays

Test no.	Toxicant addition cycle (hours on/off)	Avg. fish size (g)	Temperature (°C) avg. (Range)	pH avg. (Range)	96-h LC 50 (95% C.I.) (Conventional LC 50)		96-h MC-LC 50 (95% C.I.) (Mean concentration LC 50)		96-h PC-LC 50 (95% C.I.) (Peak concentration LC 50)	
					($mg\,l^{-1}$ NH_3-N)	($mg\,l^{-1}$ NH_3)	($mg\,l^{-1}$ NH_3-N)	($mg\,l^{-1}$ NH_3)	($mg\,l^{-1}$ NH_3-N)	($mg\,l^{-1}$ NH_3)
					Series I—*Rainbow trout*					
580*	96/0	21	9.7	7.86	31.6	0.500				
			—†	(7.81–7.89)	(26.5–37.6)	(0.420–0.593)				
574‡	6/6	18	9.6	7.86			19.3	0.292	38.2	0.579
			(9.3–9.8)	(7.79–7.97)			(16.9–22.1)	(0.257–0.332)	(33.4–43.8)	(0.510–0.658)
					Series II—*Rainbow trout*					
637	96/0	247	8.1	7.74	28.0	0.296				
			(7.9–8.3)	(7.72–7.80)	(24.8–31.6)	(0.261–0.335)				
635	6/6	230	8.3	7.74			22.3	0.221	40.4	0.401
			(8.1–8.5)	(7.64–7.84)			(19.5–25.5)	(0.195–0.251)	(35.5–45.9)	(0.356–0.453)
639	6/6	293	8.5	7.71			20.7	0.214	38.3	0.395
			(7.9–8.8)	(7.64–7.76)			NC§	NC	NC	NC
636	12/12	244	8.1	7.72			19.8	0.176	40.1	0.356
			(8.0–8.3)	(7.59–7.80)			(17.7–22.1)	(0.167–0.184)	(36.2–44.4)	(0.343–0.371)
634	6/18	230	8.2	7.72			10.5	0.099	40.0	0.377
			(8.1–8.4)	(7.61–7.84)			(7.2–15.3)	(0.067–0.149)	(28.6–56.0)	(0.266–0.536)
638¶	2.5/93.5	242	8.2	7.73					>45.3	>0.476
			—	—						
					Series III—*Rainbow trout*					
633‖	96/0	2596	7.9	7.62	21.6	0.163				
			(7.7–8.0)	(7.52–7.71)	(17.6–26.6)	(0.139–0.190)				
632‖	6/6	2080	7.7	7.67			17.0	0.145	34.2	0.292
			(7.5–8.0)	(7.60–7.83)			(14.4–20.0)	(0.122–0.173)	(29.0–40.3)	(0.246–0.347)
					Series IV—*Cutthroat trout***					
615‖	96/0	3.6	10	7.7	29.1	0.327				
					(25.3–33.4)	(0.284–0.375)				
617	96/0	4.1	10	7.7	26.3	0.296				
					(23.7–29.2)	(0.266–0.328)				
614‖	12/12	3.6	10	7.7			17.3	0.194	34.7	0.389
							(15.4–19.4)	(0.173–0.217)	(30.8–39.0)	(0.346–0.438)
616	12/12	4.1	10	7.7			19.3	0.217	38.8	0.435
							(18.4–20.3)	(0.206–0.228)	(36.9–40.8)	(0.414–0.458)

* 4 test tanks.
† — = All values measured were the same, therefore no range is given.
‡ 3 test tanks.
§ NC = Not calculable.
¶ 1 test tank.
‖ 5 fish/tank; all other other tests 10 fish/tank.
** pH and temperature values for Series IV tests are estimates.

remaining 9 fish all evidenced stress; 3 of these were floating on their backs, the other 6 either swimming on one side or rolling from side to side, and all were having difficulty breathing. These symptoms are typical of acute ammonia poisoning. The surviving 9 fish were immediately transferred to ammonia-free water, and within 30 min the 3 fish which had been floating on their backs had died; the remaining 6 fish gradually recovered their equilibrium, and all appeared to be fully recovered by the close of day 1. There were no further mortalities during the balance of the 96-h test period.

Tests continued beyond 96 h

Five of the bioassays were continued beyond the initial 96-h period, either maintaining the initial test protocol or with some variation to it.

Test 639 (6-on/6-off) was continued until day 28; there were no mortalities at the highest test tank concentration after the third cycle (during day 2) although there was one mortality each at the next two lower concentrations, one of these on day 9 and the other on day 24. The 28 day MC-LC 50 and PC-LC 50 values were the same as those for 96 h.

Test 617, a conventional bioassay, was continued through day 14 and mortalities continued to occur at all tank concentrations; no asymptotic LC 50 value was reached during the test period. The 14-day LC 50 values and their confidence intervals were 0.241 (0.229–0.254) mg l^{-1} NH_3 and 21.5 (20.4–22.6) mg l^{-1} NH_3–N. These values are 18–19% lower than the 96-h LC 50 values.

Test 616 (12-on/12-off) was also continued through day 14; however, toxicant addition was discontinued after 96 h and the fish were maintained in the test tanks without ammonia through day 14. There were no mortalities during the "recovery" period.

In test 632, following the initial 96-h cyclic period (6-on/6-off), toxicant was again raised to the peak concentrations and maintained at that level for an additional 96-h period. Four of the 5 fish at the highest tank concentration (0.37 mg l^{-1} NH_3) had expired during the first 30 h of the cyclic period; the sole survivor at 96 h also survived the sustained peak concentration for the additional 96-h period. There had been no mortalities at the next two lower concentrations (peak concentrations 0.20 and 0.15 mg l^{-1} NH_3) during the initial 96-h test period, but there was one mortality in each of these tanks within 24 h of beginning the sustained peak concentrations; the remainder of the fish in these two tanks survived the additional 96-h test period.

Test 574 (6-on/6-off) was continued with the same toxicant addition regime for 7 days beyond 96 h, but at 96 h the fish from the lower two concentrations (average peak concentrations 0.11 and 0.39 mg l^{-1} NH_3) were transferred to the highest concentration tank (average peak concentration 0.74 mg l^{-1} NH_3). Two of the 10 fish at the highest concentration and all 10 fish from each of the lower two concentrations had survived the first 96-h test period. Following transfer to the highest concentration tank 5 of the 10 fish from the lowest concentration died during the next 3 cycles; the remaining 5 fish from the lowest concentration and all 10 of the fish transferred from the middle concentration survived the additional 7-day period. The 2 fish which had already survived 96-h at the highest concentration also survived the next 7 days. Observations after day 5 included the following. During the cycle peaks, between 8–14 of the 17 fish under observation evidenced equilibrium loss, ranging from minor disorientation to floating upside down. By day 10, all but 2 fish were able to recover much of their equilibrium during the time between ammonia concentration peaks, suggesting some adaptation to the ammonia fluctuation regime. Food pellets were provided *ad libitum* starting on day 9; approximately half of the fish, including one of the fish which swam on its back, evidenced interest in eating. On day 11, toxicant addition was discontinued and fish were maintained and observed for an additional 7 days. Within 24 h most of the fish were swimming upright, although occasional lapses would occur during resting phases; this behavior continued through day 15. By day 17 all fish were feeding vigorously, appeared normal, and no loss of equilibrium was observed at any time. The test was terminated on day 18.

Mortality data treatment

In computing the MC-LC 50 and PC-LC 50 values reported above, we have taken certain liberties, as described, in our choice of data input to a conventional method for computing LC 50 values. The statistical validity of the MC-LC 50 and PC-LC 50 values we obtained has not been tested. Accordingly, the 96-h test data were further scrutinized in the following manner.

For each of the fluctuating tests (except Test 638 which had a single peak) we selected the test tank with the lowest mean ammonia concentration in which at least 50% of the test fish died. The mean concentration in that tank was compared against the LC 50 for the corresponding conventional test. Except for Test 632, the mean of the fluctuating ammonia concentrations at which 50% or more of the fish died was lower than the LC 50 value for the conventional test used for comparison (Table 3). Similarly, for each fluctuating test the tank with the highest peak ammonia concentration at which 50% or more of the test fish survived was selected, and the average peak concentration in the tank was compared against the conventional LC 50. In all cases the peak concentration values were greater than the LC 50 values from the corresponding conventional tests used for comparison (Table 3). Because two conventional tests had been conducted in Series IV, yielding two slightly different LC 50 values, the lower LC 50 value was used for comparison against the mean tank concentrations for the fluctuating tests in that series, and the higher LC 50 value was used for comparison against the

Table 3. Comparisons of conventional test LC 50 values against mean concentrations with ⩾50% mortality and peak concentrations with ⩾50% survival in fluctuating tests. (Ammonia concentrations expressed as $mg\,l^{-1}$ NH_3)

		Comparison of mean concentrations				Comparison of peak concentrations		
Test no.	Corresponding conventional test LC 50*	Lowest test tank concentration with ⩾50% mortality	% Mortality	Ratio of tank concentration to conventional LC 50	Highest test tank concentration with ⩾50% mortality	% Survival	Ratio of tank concentration to conventional LC 50
574	0.500	0.372	80	0.74	0.387	100	1.10
635	0.296	0.275	60	0.93	0.325	80	1.10
639	0.296	0.214	50	0.72	0.310	100	1.05
636	0.296	0.268	100	0.91	0.339	70	1.15
634	0.296	0.096	50	0.32	0.359	50	1.21
632	0.163	0.183	80	1.12	0.199	100	1.22
614	0.296	0.248	100	0.84	—	—	—
614	0.327	—	—	—	0.374	60	1.14
616	0.296	0.256	100	0.86	—	—	—
616	0.327	—	—	—	0.382	90	1.17

* From Table 2. See Discussion for explanation of two different LC 50 values used for comparisons with Tests 614 and 616.

peak tank concentrations. The comparisons followed the trend obtained for the other test comparisons.

Comparison of results with those reported elsewhere

Brown *et al.* (1969) tested rainbow trout in static tests in which fish were moved back and forth between tanks in which the ammonia concentrations were 0.5 and 1.5 times a previously determined 48 h LC 50. If fish were transferred on an hourly basis, the median period of survival was reported to be the same as that for constant exposure (> 700 min); if the fish were transferred at 2-h intervals, the median survival time was reported to be less (370 min). The results of the tests in which the fish were transferred at 2-h intervals are in keeping with our findings; i.e. the toxic effects from exposure to fluctuating concentrations of ammonia are greater than those from exposure to a constant concentration, if the mean "dose" exposure is the same in both cases.

CONCLUSIONS

1. Fish were better able to withstand a fixed concentration of ammonia over a 96-h time period than they were fluctuating concentrations having mean values comparable to that of the fixed concentration.
2. Fish were able to withstand short-term excursions slightly above acutely toxic (96-h LC 50) concentrations of ammonia without apparent longer-term effects, provided that those excursions were accompanied by compensatory respite periods below the acutely toxic concentrations.
3. Fish subjected for 96-h periods to cyclic pulses of ammonia at concentrations below those acutely toxic were better able to withstand subsequent exposure to acutely toxic pulses than were fish not previously so "acclimated".
4. Among the rainbow trout tested, the larger fish (> 2 kg) were more vulnerable to acutely toxic concentrations of ammonia than were the smaller fish (20–300 g).

Acknowledgements—This research was funded by the U.S. Environmental Protection Agency, Environmental Research Laboratory-Duluth, Research Grant No. R805636. Elizabeth L. Meyn and Kenneth C. Wang assisted with water chemistry analyses. Martin A. Hamilton, MSU Statistical Laboratory, contributed helpful comments with regard to the data analysis.

REFERENCES

American Public Health Association (1976) *Standard Methods for the Examination of Water and Wastewater*, 14th Edition, 1193 pp. American Public Health Association, American Water Works Association, and Water Pollution Control Federation, Washington, DC.

Brown V. M., Jordan D. H. M. & Tiller B. A. (1969) The acute toxicity to rainbow trout of fluctuating concentrations and mixtures of ammonia, phenol and zinc. *J. Fish Biol.* **1**, 1–9.

Emerson K., Russo R. C., Lund R. E. & Thurston R. V. (1975) Aqueous ammonia equilibrium calculations: effect of pH and temperature. *J. Fish. Res. Bd Can.* **32**, 2379–2383.

European Inland Fisheries Advisory Commission (1970) Water quality criteria for European freshwater fish. Report on ammonia and inland fisheries. EIFAC Tech. Paper No. 11, 12 pp; also in *Water Res.* **7**, 1011–1022 (1973).

Hamilton M. A., Russo R. C. & Thurston R. V. (1977) Trimmed Spearman–Karber method for estimating median lethal concentrations in toxicity bioassays. *Envir. Sci. Technol.* **11**, 714–719. *Correction* **12**, 417 (1978).

Metcalf & Eddy Inc. (1972) *Wastewater Engineering: Collection, Treatment, Disposal*, 782 pp. McGraw-Hill, New York, NY.

Thurston R. V., Russo R. C. & Emerson K. (1979) Aqueous ammonia equilibrium—Tabulation of percent un-ionized ammonia, 427 pp. EPA Ecol. Res. Ser. EPA-600/3-79-091. U.S. Environmental Protection Agency, Office of Research and Development, Environmental Research Laboratory-Duluth, MN.

Thurston R. V., Russo R. C. & Smith C. E. (1978) Acute toxicity of ammonia and nitrite to cutthroat trout fry. *Trans. Am. Fish. Soc.* **107**, 361–368.

U.S. Environmental Protection Agency (1974) Methods for chemical analysis of water and wastes. EPA-625/6-74-003. U.S. Environmental Protection Agency, Washington, DC.

U.S. Environmental Protection Agency (1977) Quality criteria for water, 256 pp. Office of Water and Hazardous Materials, U.S. Environmental Protection Agency, Washington, DC.

Willingham W. T., Colt J. E., Fava J. A., Hillaby B. A., Ho C. L., Katz M., Russo R. C., Swanson D. L. & Thurston R. V. (1979) Ammonia. In *A Review of the EPA Red Book: Quality Criteria for Water* (Edited by Thurston R. V., Russo R. C., Fetterolf C. M. Jr, Edsall T. A. & Barber Y. M. Jr), pp. 6–18. Water Quality Section, American Fisheries Society, Bethesda, MD.